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Element concentrations and cataract: an experimental animal model

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Summary

The determination of inorganic ions in cataractous human lenses has been the subject of several investigations; nevertheless, few studies have been concerned with trace element contents in lenses, and data are sometimes contradictory. An animal experimental model of induced cataract is here proposed with the aim of evaluating the changes of Ca, Na, K, Cu and Zn concentrations. The cataract was produced by an Nd: YAG Laser treatment of the right eye of sexteen male rabbits. The determination of the elements was performed by atomic absorption spectrometry (both flame and flameless methods) after an acid digestion of samples. Compared with the results obtained in left lenses used as a control (Ca 14.4±5.7 mg/kg d.w.; Na 1.3±0.5 g/kg d.w.; K 9.9±1.1 g/kg d.w.; Cu 0.24±0.09 mg/kg d.w.; Zn 24.8±2.3 mg/kg d.w.), the mean concentration values of opaque lenses showed some significant changes for Ca, Na, and Cu (Ca 123.7±106.6 mg/kg d.w.; Na 4.5±4.3 g/kg d.w; Cu 0.43±0.21 mg/kg d.w.). Potassium showed a tendency to decrease, and zinc to increase. Positive correlations were found between calcium and sodium both in controls ($r = 0.73$, $p < 0.001$) and in treated lenses ($r = 0.87$, $p < 0.0001$). An inverse correlation between Ca and K confirmed the tendency of potassium to decrease.

Keywords: Human lenses, cataract, trace elements.
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Introduction

The determination of inorganic ions such as Ca, K, Mg and Na in clear and cataractous lenses has been the subject of several studies (1-3) carried out with the aim of evaluating the role these ions have in the mechanism of cataractogenesis. The data showed that the formation of senile cataract is strictly associated with the impairment of the permeability of the eye lens membrane, resulting from the accumulation of calcium and sodium ions, and the loss of potassium (4-5). These observations have been also confirmed in a previous study by the present authors (6). The information reported in literature regarding the levels of trace elements in cataractous lenses are in part contradictory and the effective role of inorganic elements still needs to be clarified. For this reason, the involvement of various metals in cataract formation is currently an area of intensive research.

The aim of the present work was to evaluate whether an animal model of induced cataract would cause changes in the mineral content of lenses. This would represent a good way of studing the behaviour of the elements in cataractous lens, overcoming at the same time ethical aspects and the difficulty of obtaining normal human lenses to be used as controls. Therefore, treatment with a laser was used to produce a lenticular photofragmentation and, consequently, large lens opacities; the right eye of each rabbit was treated, while the left eye was preserved clear as control. The concentration of Ca, Na, K, Cu and Zn was measured both in clear and cataractous eye lenses by flame (Ca, Na, K, Zn) and graphite furnace (Cu) atomic absorption spectrometry (FAAS and GFAAS, respectively). The elements investigated were chosen because of their role in ocular tissues.

Role of Ca, Na, K, Cu and Zn in the eye

Calcium plays an important role in muscular contraction, participating in mechanisms which involve numerous substances, such as myosin and actin (7). These two compounds have

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been observed in both corneal epithelium and endothelium. Calcium is also necessary for calpain and calmodulin reactivity. In lenses, the concentration of Ca represents an important factor for the permeability of the membrane to Na ions (8); moreover, Ca might take part in the transformation of soluble proteins into insoluble ones. Maintenance of calcium homeostasis is imperative for clarity of the lens, and Ca_2+-ATPase is essential for removal of cytosolic calcium. Membranes are deranged in cataractous tissue, which should lead to altered levels of Ca (9). A prolonged increase of the element concentration would be expected to activate proteases, such as calpain, and so could induce unexpected and irreversible breakdown of important structural proteins (10). Sodium and potassium participate in the metabolic processes of the lens by activating (K) or inhibiting (Na) the corresponding enzymes. Sodium also attracts water and Cl ions, thus disturbing the osmotic balance (2). Zinc is present in relatively high concentrations in the choroid and the retina. It is believed that its main function in the eye is to keep the retina in position. Zinc also plays an important role in vision as it actives the retinene reductase enzyme (11). Many enzymes contain zinc and their activity decreases as senile cataract develops; on the otherhand, high levels of zinc in traumatic cataract indicate a moderate disturbance of crystalline lens metabolism, as compared with senile cataract (12). The presence of Cu in lens tissue is metabolically required for both transparency and the proper functioning of the cytochrome oxidase and coenzyme A-dehydrogenase systems. It also plays an important role in the oxidation scavenging reactivity of Cu, Zn-SOD.

Materials and Methods

Housing and treatment of animals

Sixteen male, twenty- week old, New Zealand rabbits, supplied by Gennari (Rieti, Italy), weighing 3.2±0.38 kg, were housed according to the EEC 609/86 Directive regulating the welfare of animals. All the rabbits were checked before starting the experiment to exclude the presence of natural opacity in the lenses, and were monitored clinicaly through out the study.

The right eye of each rabbit was treated with a Nd: YAG laser (Microruptor II). The laser parameters applied were: energy 28.3 mJ/pulse; no.of pulses/spot: 2; no. of spots: 35. The treatment was performed under total anaesthesia induced by intramuscular injection of Chloropromazine (Largactil 0.5 ml), Ketamine (Ketalar 50 mg, 3 ml), and 2% Xilocaine (1 ml). The photofragmentation was performed from the lower sectors of the posterior cortex towards the nucleus and the anterior cortex. During treatment, special attention was paid to avoid damage to the anterior or posterior capsule of the lenses; none of treated animals showed signs of phlogosis of the anterior segment or increase im ocular pressure. The left eye of each rabbit was preserved as as individual control. After treatment the animals were housed for a further 45 days.

Lens collection

All animals were sacrificed under anaesthesia by cardiac puncture (Tanax) and the eye lenses were carefully dissected from the globe, avoiding contamination. The specimens were immediately transferred to polyethylene vials (pre-treated with nitric acid 1% v/v and ultrapure water), and frozen at -20° C until analysis.

Sample treatment

Prior to analysis, the lenses were dried at 103±2°C to determine the water content. Each sample was initially digested in a quartz tube on a hot plate thermoregulated at 100°C, using 2 ml of concentrated HNO_3. After lens dissolution, the temperature of the hot plate was increased to 150°C in order to evaporate the solution to about half of its volume; this step required about 15-20 min. Two further aliquots (2 ml) of HNO_3 were added until a colourless solution was obtained. After most of the acid had evaporated and approximately 200 ml remained in the bottom of the tube, the digestion was stopped to prevent evaporation to dryness. After cooling, the volume of the solution was increased to 3 ml with high-purity water. To avoid contamination, all the operations were performed under controlled conditions (clean room).

Reagents and standard solutions

All reagents used in the study were of Suprapur type (Merck, Darmstad, Germany). Calibrants for the determination of elements were prepared daily from certified 1 g/l Merck commercial solutions. ASTM type I high-purity water was used with a background resistivity of not less than 16.6 megaohms-cm (≤ 0.06 µs/cm) (Labconco, USA).

Instrumental

The determinations of Ca, Na, K, and Zn were performed using the air-acetylene FAAS technique, whereas the GFAAS technique was employed for the analysis of Cu. A Perkin-Elmer 5100 Zeeman spectrometer was used in both flame and flameless modes. In the first method, the background correction was obtained by continuum source correction systems (deuterium and tungsten lamps). In the flameless measurements a HGA 600 graphite furnace and an AS-60 autosampler were employed. The calcium, sodium and potassium determinations were performed in a 0.1% lanthanium solution to prevent either phosphate or ionization interferences. In the analysis of copper, matrix modification was obtained by means of addition of

$NH_4H_2PO_4$ (5µl of a 60 g/L solution). The quantification was done against a calibration curve; nevertheless, the standard addition method was also employed in order to verify the validity of the calibration procedure. In all measurements, the sample dilution ratio was made so as to remain within the linear range of the analytical response. All determinations were made in triplicate and the results averaged. The instrumental parameters adopted are reported in Tables 1-2.

Data on quality control tests

In order to evaluate recovery and precision of the analyses, some clear rabbit lenses not included in the analytical protocol were mixed to form a larger sample. Sub-samples were spiked with suitable amounts of the elements investigated. The mean recoveries were: Ca, 97.2%; Na, 96.4%; K, 94.1%; Cu, 93.7%; Zn, 107%. The coefficients of variation, with respect to the entire procedures, were: Ca, 7%; Na, 6%; K, 7%; Cu, 8%; Zn, 5%. In addition, a SRM 909b (lyophilized serum) reference material from NIST, certified for Ca, Na and K, was also analyzed using the same procedure as applied to the samples. The accuracies were: Ca, 97.7%; Na, 97.4%; K, 96.8%. The detection limits (whole procedure) were (mg/kg): Ca 0.9; Na 2.5; K 2.5; Cu 0.009; Zn 1.5.

Statistical analysis

The statistical analysis involved several tests, such as the analysis of variance, Student's t-test for paired data, the Kolmogorov-Smirnov test, and matrix correlation.

Results and discussion

All the animals survived the experiment and developed varying degrees of opacity of the crystalline lenses as early as ten-days after treatment. Nevertheless, housing of the animals for a further 45 days permitted a complete evolution of confluent opacities both cortical and nuclear; none of the controls developed opacity until . The energy of the YAG laser treatment was quite high when compared to the energy clinically used, but this was necessary to obtain the effect expected on the lenses. The energy was experimentally chosen by gradually increasing its value until a lesion appeared (a large blister of cavitation).

The general results obtained for Ca, Na, K, Cu, and Zn are reported in Table 3. Data are expressed on the basis dry weight. Table 4 reports mean concentration values both for treated eyes and controls. The values of blanks, calculated for each sample, resulted always close to or below the detection limits of the method (whole procedure).

Table 1. Ca, Na, K, and Zn determinations: FAAS analysis parameters

Element	Wavelength (nm)	Slit (nm)	Airflow (L/min)	C_2H_2flow (L/min)	Sensitivity (mg/L)
Ca	422.7	0.7	2.5	9.0	0.09
Na	589.0	0.4	2.2	9.5	0.01
K	766.5	1.4	2.5	7.5	0.04
Zn	213.9	0.7	3.0	9.0	0.02

Table 2. Cu GFAAS determination: furnace parameters

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Gas Flow mL/min
Drying I	160	10	10	300
Drying II	250	10	10	300
Ashing	900	20	10	300
Atomizing	2550	0	5	0
Cleaning	2650	1	5	300
Cooling	20	1	10	300

Gas Flow: Argon UPP. Tube: pyrocoated with l'Vov platform,, Wavelenght 324.8 nm, Slit: 0.7 nm, Sensitivity (characteristic mass): 8.0 pg/ 0.0044 A-s)

Table 3. Ca, Na, K, Cu and Zn content (d.w.) in normal and opaque lenses of rabbit

Rabit n°	Ca mg/Kg.	Na g/Kg	K g/Kg	Cu mg/Kg	Zn mg/Kg
1R	179	6.0	8.3	0.40	25.8
1L	10.5	0.8	9.5	0.18	23.5
2R	55.6	1.3	11.1	0.33	25.0
2L	11.2	1.1	9.2	0.09	28.1
3R	79.7	1.6	8.0	0.49	23.4
3L	15.5	1.3	9.2	0.32	23.9
4R	301	15.9	9.4	1.02	28.3
4L	6.5	0.9	13.4	0.28	28.1
5R	48.5	1.5	8.7	0.32	26.2
5L	17.5	1.4	9.2	0.27	26.2
6R	71.9	8.3	9.0	0.44	24.1
6L	8.8	1.3	9.2	0.79	29.2
7R	280	8.4	2.4	0.17	20.3
7L	21.6	1.8	9.3	0.26	19.7
8R	82.0	2.2	7.3	0.45	25.5
8L	15.1	1.5	9.0	0.41	23.4
9R	14.2	1.2	10.5	0.27	28.9
9L	25.5	2.1	10.4	0.29	25.5
10R	98.9	1.7	10.9	0.43	27.7
10L	16.1	2.7	10.8	0.24	25.8
11R	320	10.1	7.2	0.46	21.8
11L	8.7	0.7	9.6	0.09	23.6
12R	43.4	1.2	9.8	0.31	25.9
12L	12.8	1.0	10.0	0.21	25.3
13R	247	9.0	8.0	0.62	25.2
13L	11.7	0.9	9.8	0.23	24.6
14R	20.0	0.9	9.5	0.25	24.7
14L	25.2	1.9	9.8	0.32	23.8
15R	16.0	0.9	11.1	0.22	24.6
15L	13.9	1.2	10.4	0.23	23.9
16R	122	2.1	12.1	0.67	28.3
16L	10.3	0.8	10.3	0.23	23.7

As for control specimens, the concentrations of potassium and zinc showed a coefficient of variation close to that of the analytical methods, whereas calcium, sodium and copper exhibited a greater variance, probably due to biological differences. Within the variability observed, a highly significant correlation ($r= 0.73$, $p< 0.001$) was found between Na and Ca in controls (Figure 1); this correlation is explained by a direct interdependence between these two elements and the differences in lens membrane potential (13,14). The concentration of the elements studied showed the same order of magnitude as that reported for human lenses (6, 13,14).

With exception of zinc, large values of standard deviation were generally observed in opaque lenses (e.g. Ca≈ 86%) and they were statistically higher than those calculated for normal lenses; this could be attributable to a different individual response to the treatment.

In spite of the large standard deviation values generally observed in opaque lenses, significant changes in the concentrations of some elements occurred after laser treatment (Tab.4). Calcium, sodium and copper concentrations significantly increased (Ca: $p<0.001$; Na: $p<0.01$; Cu: $p<0.04$),. In particular, calcium and sodium in opaque lenses were higher than in controls by a factor of 8.6 and 3.5, respectively, copper was higher by a factor of 1.8.

A significant correlation between Na and Ca also occurred in treated lenses and was characterized by better parameter values ($r= 0.87$, $p< 0.0001$) (Figure 2).

Non-significant differences between treated and control lenses were observed both for zinc and potassium; however, the concentration of potassium showed a tendency downwards, also suggested by a weak inverse correlation observed between calcium and potassium ($r= -0.56$, $p< 0.02$) (Figure 3).

Our results are mostly in agreement with those reported by other authors (2, 4-6, 10) who determined the same elements both in cataractous and normal human lenses. In general, among the elements studied, calcium was most strongly influenced by the treatment. This finding agrees with research carried out by other authors (14-16) who had observed that calcium

Table 4. Mean concentration (d. w.)values of Ca, Na, K, Cu and Zn in normal and opaque lenses

	Normal lenses	Opaque lenses	p<
Ca (mg/kg)	14.4 ± 5.7	123.7 ± 106.6	0.001
Na (g/kg)	1.3 ± 0.5	4.5 ± 4.3	0 .01
K (g/kg)	9.9 ± 1.	8.9 ± 2.3	n.s.
Cu (mg/kg)	0.24 ± 0.09	0.43±0.21	0.04
Zn (mg/kg)	24.8 ± 2.3	25.3 ± 2.3	n.s.

d.w. = dry weight; n.s. = not significant

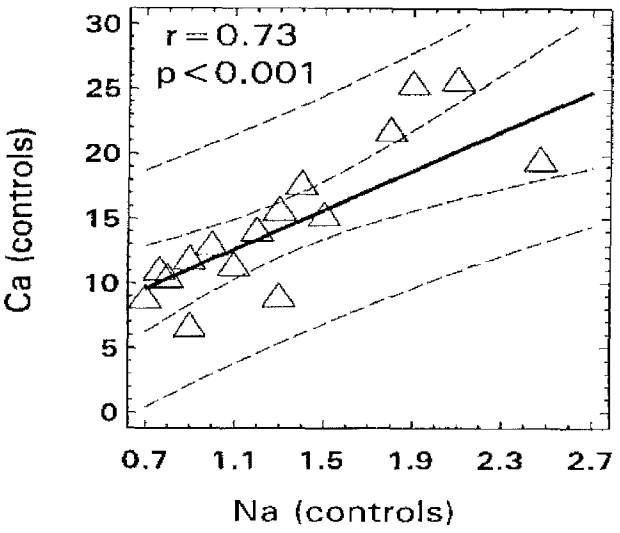

Figure 1. Regression of calcium (mg/Kg d.w.) and sodium(g/Kg d.w.)and sodium(g/Kg d.w.) in controls.(Dashed lines show confidence intervals of 95% and 99%).

is an element particularly involved in the mechanism of opacity formation in the process of cataractogenesis. Furthermore, they noticed that an increase of Na and a loss of K do not produce lens opacity if the calcium concentration remains within normal values; on the otherhand, an increase in calcium concentration, coupled with an imbalance of Na and K, brings about a pathological condition.

From the analytical point of view, the method adopted was suitable for this kind of study. The procedure of sample digestion is easy and does not require complex equipment. As regards copper determination, the results calculated by means of a calibration curve did not differ statistically from those obtained by applying the standard addition method, which means that the

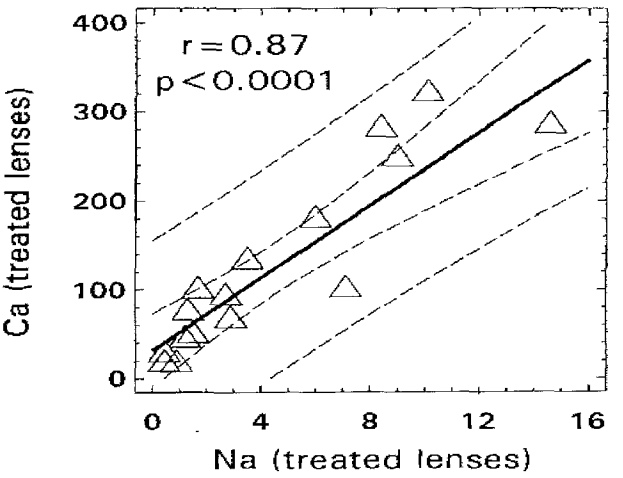

Figure 2. Regression line of calcium (mg/Kg d.w.) and sodium (g/Kg d.w.) in treated lenses. (Dashed lines show confidence intervals of 95% and 99%).

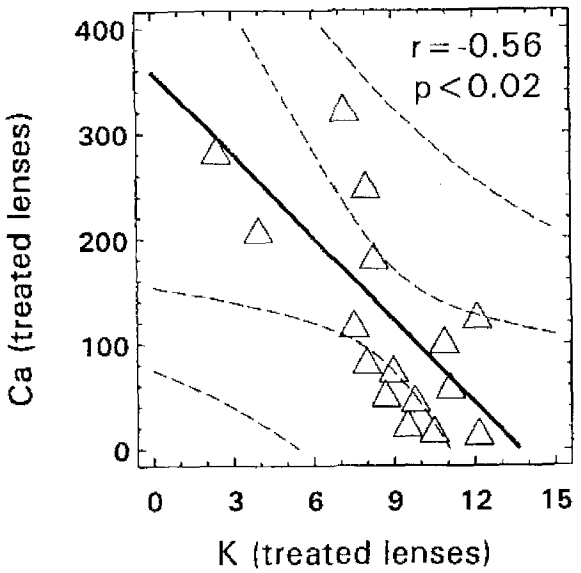

Figure 3. Regression line of calcium (mg/kg d.w.) and Potasium (g/kg d.w.) in treated lenses. (Dashed lines show confidence intervals of 95% and 99%).

choice of matrix modifier and furnace instrumental setting was correct.

Conclusions

The results indicate that the experimental model proposed can be used to induce lens opacity in animals, that it is suitable for studying aspects of cataractogenesis and, at the same time, provides both control and treated specimens from the same individual. Therefore, as compared with other animal protocols (17,18) based on cataracts induced by administration of chemical substances (e.g., Na_2SeO_3), it offers the advantage that results are less influenced by biological variability. Moreover, our model overcomes the ethical aspects entailed by the use of human material for experiments, and the great difficulty in finding transparent lenses to be used as controls.

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References

1) JEDZIMAK, J.A; NICOLI, D.F; YATES, E.M. AND BENEDEK,G.B. (1976).
On the calcium concentration of cataractous and normal human lenses and protein fractions of cataractous lenses. Exp. Eye Res., 23 , 325-332.
2) STANOJEVIC-PAOVIC, A.; HRISTIC, V.; CUPERLOVIC, M; JOVANOVIC, S. AND KRSMANOVIC, J. (1987). Macro and microelements in the cataractous eye lens. Opthalmic Res., 19, 230-234.
3) RINGVOLD, A; SAGEN, E; BJERVE, K.S. AND FØLLING, I. (1988). The calcium and magnesium content of the human lens and aqueous humor. Acta Opthalmol., 66 , 153-156.
4) XIAOLIN HOU, AND YUHUI HOU. (1996) . Determination of 19 elements in human eye lenses. Biol. Trace Element Res., 55, 89-98.
5) SHUKLA, N; MOITRA, J.K.; TRIVEDI, R.C. (1996). Determination of lead, zinc, potassium, calcium, copper and sodium in human cataract lenses. Sci. Total Environ., 181, 161-165.
6) GIORDANO, R.; COSTANTINI, S.; VERNILLO, I.; MORAMARCO, A.; RASI, V.; GIUSTOLISI, R. AND BALACCOGABRIELI, C. (1992). Calcium,potassium, zinc, and copper determinations in cataractous human lenses. Microchem. J., 46, 184-190.
7) COSTANTINI, S.; GIORDANO, R. AND PESCOSOLIDO, N. (1981). Gli elementi inorganici nel film lacrimale di soggetti sani. Nota I: Li, Na, K, Ca, Mg. Ann. Ottalmol. Clin. Ocul., 107, 755-765.
8) HIGHTOWER, K.R. AND REDDY, V.N. (1982). Calcium-induced cataract. Invest. Opthalmol. Vis. Sci., 322, 263-267.
9) PATERSON, C.A; ZENG, J; HUSSEINI, Z; BORCHMAN, D; DELAMERE, N.A;
GARLAND, D; JIMENEZ-ASENSIO, J. (1997). Calcium ATPase activity and membrane structure in clear and cataractous human lenses.Current Eye Res., 16/4, 333-338.
10) DUNCAN, G. AND WORMSTONE, I..M. (1999). Calcium cell signalling and cataract: role of the endoplasmic reticulum. Eye, 13/3, 480-483.
11) COSTANTINI, S; PESCOSOLIDO, N. AND GIORDANO, R. (1983). Gli elementi inorganici nel film lacrimale di soggetti sani. Nota III: Cu, Zn, Co, Ni. Ann.Ottalmol. Clin. Ocul, 109, suppl. 6, 631-642.
12) JERU, I. (1997). Rolul zincului in aparictia cataractei. Oftalmologia, 41(4), 329-332.
13) DUNCAN, J. AND BUSHELL, A.R. (1975). Ion analyses of human cataractous lenses. Exp. Eye. Res., 20, 223-228.
14) RACZ, P. AND ORDOGH, M. (1977). Investigation on trace elements in normal and senile cataractous lenses. Albrecht v. Graefes Arch. klin. Exp. Ophthal., 204,67-72.
15) CEKIC, O. (1998). Copper, lead, cadmium and calcium in cataractous lenses. Ophtalmic Res., 30, 49-53.
16) HIGHTOWER, K.R. AND HARRISON, S.E. (1982). Valinomycin cataract: the relative role of calcium and sodium accumulation. Exp. Eye Res., 34, 941-943.
17) PALMQUIST, B.M; FAGERHOLM, P. AND LANDAU, I. (1986). Selenium-induced cataract. A correlation of dry mass content and light scattering. Exp. Eye Res., 42. 35-42.
18) HU ZHAOHUI; ZHANG JAIPING; LUI SHIRONG; ZHONG. MIN. AND ZHANG CHANGJING. (1990). Study of trace-element metabolism during the formation of rat's cataract. J. Radianalyt. Nuclear Chem., Articles, 141, 1, 155-162.